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GASTROINTESTINAL STROMAL TUMORS – PATHOGENETIC MECHANISMS, PHENOTYPIC CHARACTERIZATION AND PROGNOSIS

Akademisk avhandling


av

Johanna Andersson

Fakultetsopponent: Professor Olle Larsson,
Institutionen för onkologi-patologi, Enheten för patologi,
Karolinska Institutet, Stockholm

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GASTROINTESTINAL STROMAL TUMORS – PATHOGENETIC MECHANISMS, PHENOTYPIC CHARACTERIZATION AND PROGNOSIS

Johanna Andersson

Lundberg Laboratory for Cancer Research, Department of Pathology, Sahlgrenska Academy at Göteborg University, Sahlgrenska University Hospital, SE-413 45 Göteborg, Sweden

Gastrointestinal stromal tumor (GIST), the most common non-epithelial neoplasm of the gastrointestinal tract, has historically been problematic both conceptually and clinically. Recently, GIST has been shown to share phenotypic features with the interstitial cells of Cajal (ICC), including the almost uniform expression of the tyrosine kinase receptor KIT. GIST have frequently been found to have activating mutations of the KIT gene or more rarely the platelet derived growth factor receptor alpha (PDGFRα) gene; these apparently play a key role in GIST’s pathogenesis. The occurrence of GIST lacking KIT and PDGFRα mutations indicates, however, that alternative pathogenetic mechanisms exist.

Clinical studies regarding the correlation of mutations with disease outcome in GIST have been contradictory. Mutation analysis of the KIT gene in 14 GIST revealed exon 11 mutations in 9 tumors. Mutations were detected in benign as well as malignant GIST. Cytogenetic and SKY analyses revealed several recurrent structural and numerical abnormalities; losses of 1p, 14 and 22 being the most common. No correlation was found between biologic behavior, KIT mutation status and chromosome aberrations. All tumors preferentially expressed the shorter, tumorigenic, splice variant of exon 9 of the KIT gene.

Imatinib mesylate, a new designer drug, selectively inhibits type III receptor tyrosine kinases and has a dramatic, anti-tumor effect on GIST. In a center-based study of 17 patients with GIST, we studied the relationship between KIT exon 11 mutation status and treatment response. Imatinib was especially effective in tumors with exon 11 mutations - KIT exon 11 mutations were detected in 8 of 9 patients with partial response, whereas no mutations were detected in 3 patients with stable or progressive disease.

Earlier studies of receptor tyrosine kinase mutations indicate that different types of mutations are associated with distinctive phenotypes and potentially clinical behavior in GIST. In a large, retrospective, population-based series of GIST from the pre-imatinib mesylate era, we examined whether mutation type correlated with phenotype and clinical course. KIT exon 11 mutations were detected in GIST from 117 of 233 patients (69 deletions, 27 missense mutations and 21 duplications). The deletion subgroup had a significantly decreased disease-free survival. KIT exon 11 deletions were found to be an independent adverse prognostic factor with respect to disease-free survival.

GIST have been reported to occasionally occur in patients with neurofibromatosis type I (NF1). NF1 is the most common autosomal dominant inherited disorder, with an incidence of 1/3-4000 individuals. The clinical spectrum of NF1 is extremely wide and includes organic as well as mental manifestations. We analyzed GIST arising in 15 NF1 patients and found that this subset of GIST has a unique phenotype, including an almost uniformly benign appearance histologically, a propensity for multicentricity, association with ICC hyperplasia, predominant location in the small intestine, and a spindle cell morphology as well as benign or very low grade malignant behavior. The NF1-associated GIST also have a unique genotype in that they lack KIT and PDGFRα mutations, indicating that different pathogenetic mechanisms are involved in their evolution.

In summary, we have shown that the role of KIT mutations in the biological behavior of GIST is more complex than previously recognized and that the type of mutation influences clinical behavior as well as response to imatinib treatment. NF1-associated GIST are a pheno-and genotypically unique subset of GIST that lack activating mutations in the KIT and PDGFRα genes.

Key words: Gastrointestinal stromal tumor (GIST), neurofibromatosis type I (NF1), mutation, prognosis, denaturating high performance liquid chromatography (dHPLC), sequencing, KIT, PDGFRα, NF1

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2005
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fortsätt längta
hitta mod
våga hoppas
våga tro
tro på drömmen
ta ett kliv
välj den värld du lever i
och var en galning
var naiv
det är det som håller oss vid liv

/Peter LeMarc, "Det som håller oss vid liv"
ABSTRACT

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ISBN 91-628-6431-9
LIST OF PUBLICATIONS

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ABBREVIATIONS

ABL  Abelson murine leukemia viral oncogene
ACN  Acetonitrile
ATP  Adenosine triphosphate
BCR  Breakpoint cluster region
bp   base pair
CD17 KIT protein product
cDNA Complementary DNA
CML  Chronic myelogenous leukemia
dHPLC Denaturing high performance liquid chromatography
DNA  Deoxyribonucleic acid
DVB  Divinyl benzene
GAP  Guanosine triphosphate activating protein
GDP  Guanosine diphosphate
GTP  Guanosine triphosphate
gDNA genomic DNA
GANT Gastrointestinal autonomic nerve tumor
GIPACT Gastrointestinal pacemaker cell tumor
GIST Gastrointestinal stromal tumor
hpf  high power field
ICC  Interstitial cells of Cajal
kb   kilo base pair
kDa  kilo Dalton
Ki67 nuclear antigen expressed in proliferating cells
KL   Kit ligand
LOH  Loss of heterozygosity
MAPK Mitogen activated protein kinase
MGF  Mast cell Growth factor
mRNA messenger RNA
NF1  Neurofibromatosis type 1
PCR  Polymerase chain reaction
PDGFRα Platelet derived growth factor receptor alpha gene
PDGFRα Platelet derived growth factor receptor alpha protein
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
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<tbody>
<tr>
<td>PI3K</td>
<td>Phosphatidylinositol 3-kinase</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RTK</td>
<td>Receptor tyrosine kinase</td>
</tr>
<tr>
<td>SCF</td>
<td>Stem cell factor</td>
</tr>
<tr>
<td>SF</td>
<td>Steel factor</td>
</tr>
<tr>
<td>SKY</td>
<td>Spectral karyotyping</td>
</tr>
<tr>
<td>STAT</td>
<td>Signal transducer and activator of transcription</td>
</tr>
<tr>
<td>TEAA</td>
<td>Triethylammonium amine</td>
</tr>
<tr>
<td>TP53</td>
<td>Tumor protein 53</td>
</tr>
<tr>
<td>WT</td>
<td>Wild type</td>
</tr>
</tbody>
</table>
INTRODUCTION

Genetic basis of cancer

Over the past few decades, cancer has been shown to be a genetic disease. Its development is a multi-step process characterized by the stepwise transformation of a normal cell into a tumor cell.\(^1\)\(^2\) During cancer development, somatic cells accumulate a number of genetic changes that lead to uncontrolled cell growth, loss of differentiation, and frequently increased motility.\(^3\) Through these changes, which may be subtle, as in point mutations, deletions and insertions, or which may involve chromosomal rearrangements, such as gains and losses, amplifications and translocations, the cell acquires the necessary functional capabilities to become a fully transformed malignant tumor cell. In a landmark publication, Hanahan & Weinberg\(^4\) described six such functional capabilities believed to be essential for malignant neoplasms, namely: self sufficiency in growth signals; insensitivity to anti-growth signals; limitless replicative potential; evasion of apoptosis; sustained angiogenesis; and tissue invasion and metastasis.

The majority of genes that are responsible for or control these capabilities can be divided into the three major classes of cancer genes, \textit{i.e.} oncogenes, tumor suppressor genes and DNA repair genes.\(^5\)

\begin{itemize}
  \item \textbf{Oncogenes}
  \begin{itemize}
    \item Proto-oncogenes are normal genes that are often involved in the control of cell growth and proliferation, processes that are normally strictly regulated in the cell. Conversion of normal proto-oncogenes into activated oncogenes is an important step in the tumorigenesis of many tumors. Oncogenes are activated by dominant, “gain-of-function” mutations and consequently only one allele is normally affected by the mutation. They may be divided into different classes or families depending on the function of the proteins they encode, such as growth factors, growth factor receptors and signal transduction molecules. Several receptor tyrosine kinases act as oncogenes, including KIT and PDGFR\(\alpha\).
  \end{itemize}

  \item \textbf{Tumor suppressor genes}
  \begin{itemize}
    \item Tumor suppressor genes are also known as the gatekeepers of the cell and they function as negative regulators of cell proliferation, encoding cell cycle inhibitors, pro-apoptotic factors and transcriptional repressors. The mutations that affect tumor suppressor genes are recessive, “loss-of-function” mutations; both the maternal and the paternal alleles must be inactivated in order for these genes to contribute to tumorigenesis as postulated by Knudson in the “two-hit” hypothesis published in 1971.\(^6\) Patients with hereditary forms of cancer often inherit a germline mutation in one of the alleles of a tumor suppressor gene.
  \end{itemize}

  Mutations of \textit{TP53} are among the most common aberrations known to occur in human cancers, sporadic as well as hereditary. For example, patients suffering from the Li Fraumeni syndrome have germline mutations in this tumor suppressor gene and are
predisposed to develop various malignancies including soft tissue sarcomas. TP53, perhaps the most well-known tumor suppressor gene in the human genome, encodes a protein that mediates cell cycle arrest and promotes apoptosis in response to DNA damage.\textsuperscript{7-9} Inactivation of TP53 prevents the cell from undergoing DNA-damage induced cell cycle arrest or apoptosis and leads to genetic instability due to the accumulation of mutations.\textsuperscript{10}

**DNA repair genes**

The third class of cancer associated genes includes the DNA repair genes ("caretakers"). These genes encode proteins that maintain the integrity of the genome. Loss of this function, due to mutations, results in genomic instability.\textsuperscript{11,12} There are several mechanisms for DNA repair, including direct repair, base excision repair, and mismatch repair (reviewed in Lehmann, 1995).\textsuperscript{13,14} Defects in the DNA repair mechanisms indirectly increase the risk of cancer development by increasing the mutation rate.

**Gastrointestinal cancer**

By far the most common type of gastrointestinal cancer is adenocarcinoma, particularly involving the colon, rectum and stomach. After breast, lung, and prostate cancer, colorectal cancer is the most common malignant tumor occurring in the western world. Malignant tumors of the small intestine are rare and account for less than 2\% of all gastrointestinal cancers.\textsuperscript{15,16}

Malignant mesenchymal tumors (sarcomas) of the gastrointestinal tract are relatively uncommon. The National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) data from 1995 report that sarcomas account for 2.2\% of gastric malignancies, 13.9\% of small bowel malignancies and only 0.1\% of all colorectal malignancies.\textsuperscript{17}

**Gastrointestinal stromal tumors (GIST)**

**Clinical presentation and prognosis of GIST**

Gastrointestinal stromal tumors (GIST), the focus of this thesis, are the most common mesenchymal (non-epithelial) neoplasms of the gastrointestinal tract. A recent population-based study of GIST in western Sweden has indicated a yearly incidence of 14.5/10\textsuperscript{6} inhabitants and a prevalence of 129/10\textsuperscript{6} inhabitants.\textsuperscript{18} GIST typically occur in middle aged and older individuals; in the population-based study of 288 GIST in western Sweden, the median age was 69 years (range 10-92 years).\textsuperscript{18} Most studies show an equal gender distribution.\textsuperscript{18,20} Tumor sizes range from 2 – 30 cm at clinical presentation. The majority of tumors are detected due to symptoms but approximately 30\% are incidental findings at surgery or autopsy. In the population-based study of 288 GIST in western Sweden, 69\% of the tumors were symptomatic, causing abdominal pain, gastrointestinal bleeding, gastrointestinal obstruction or a palpable mass.\textsuperscript{18} The stomach is the most common site (50-70\%) for GIST, followed by the small intestine (20-30\%), and colon-
rectum (<10%). More rarely, GIST occur in the esophagus or in other sites such as the omentum or the mesentery (less than 5%).

There has been much confusion and controversy regarding the prognostication of GIST. Previously, a substantial proportion of GIST have been regarded as benign. Recently, however, most GIST, including very small, incidentally detected tumors, have been shown to be capable of metastasis. In 2001, a consensus risk stratification system for GIST was formulated based on expert opinions. This system classify GIST according to their malignant potential; based on size and mitotic activity, the tumors are divided into four different risk groups, as shown below:

<table>
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<tr>
<th>Risk group</th>
<th>Size (cm)</th>
<th>Mitotic count</th>
</tr>
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<tbody>
<tr>
<td>Very low risk</td>
<td>&lt;2</td>
<td>&lt;5/50hpf</td>
</tr>
<tr>
<td>Low risk</td>
<td>2-5</td>
<td>&lt;5/50hpf</td>
</tr>
<tr>
<td>Intermediate risk</td>
<td>&lt;5</td>
<td>6-10/50hpf</td>
</tr>
<tr>
<td></td>
<td>5-10</td>
<td>&lt;5/50hpf</td>
</tr>
<tr>
<td>High risk</td>
<td>&gt;5</td>
<td>&gt;5/50hpf</td>
</tr>
<tr>
<td></td>
<td>&gt;10</td>
<td>Any mitotic rate</td>
</tr>
<tr>
<td></td>
<td>Any size</td>
<td>&gt;10/50hpf</td>
</tr>
</tbody>
</table>

Overtly malignant* Metastasis at presentation


In the population-based study of 288 GIST in western Sweden, Nilsson and co-workers found that 63% and 83% of high risk and overtly malignant GIST were associated with tumor related deaths (median follow-up intervals, 40 months and 16 months, respectively) compared to only 2 of 170 (1.2%) patients with very low, low or intermediate risk GIST. The authors proposed a refined prognostication system for GIST based on tumor size and proliferative index. The maximum tumor size (in centimeters) and the maximum Ki67 proliferative index (in percentage) are added to give a risk score. Risk scores ≥ 7 are associated with a decreased 5 year survival; the likelihood of dying increases rapidly with higher risk scores. This risk score system is simpler and therefore probably more reproducible than other existing prognostication systems, further stratifies risk groups, and is potentially more useful in the evaluation of treatment protocols.

The evolving concept of GIST

GIST have a wide morphological spectrum, ranging from small clinically benign lesions with a bland histologic appearance to large, highly malignant tumors with spindled and/or epithelioid cellular features. Problems regarding criteria for diagnosis, appropriate nomenclature, identification of reliable clinical and morphologic prognostic factors, and
type of differentiation have existed for decades. Conflicting views regarding their differentiation, classification and prognosis have been reflected by a variety of names used for these tumors. In the past, they were usually believed to be of nerve or nerve sheath origin. Later, most mesenchymal tumors of the gastrointestinal tract were thought to be of smooth muscle origin, since they histologically had some smooth muscle-like features, thus terms such as leiomyoma, leiomyoblastoma, and leiomyosarcoma, sometimes with the prefix “epithelioid”, were commonly used. In the 1980s and early 1990s, however, immunohistochemical and ultrastructural studies showed that true smooth muscle tumors are very rare in the gastrointestinal tract. The majority of gastrointestinal mesenchymal tumors differ from true smooth muscle tumors of other sites in that they rarely express desmin and smooth muscle actin and ultrastructurally lack well-developed myofilaments. In 1983, Mazur and Clark published a paper documenting the lack of smooth muscle differentiation, which led to a significant nomenclature change. They proposed the term “stromal tumors”, later modified to “gastrointestinal stromal tumor” (GIST) to reflect that these tumors display different lines of differentiation rather than true smooth muscle. The use of immunohistochemical and ultrastructural techniques to classify GIST also led to the recognition that a subset of these tumors express some features of nerve cell differentiation, and in 1984, Herrera and co-workers introduced the term “plexosarcoma”. This subset of GIST was later labeled as gastrointestinal autonomic nerve tumors (GANT).

Figure 1. Schematic illustration of the evolving concept and terminology of GIST. (Adapted from Chan, 1999)
Recent breakthroughs in the understanding of GIST

Recent studies have demonstrated a close phenotypic resemblance between GIST and the interstitial cells of Cajal (ICC).\textsuperscript{30,31} In 1998, Kindblom and co-workers demonstrated striking ultrastructural similarities between the ICC and GIST (78 cases); they also found that GIST, similar to the ICC, strongly expressed the KIT receptor tyrosine kinase.\textsuperscript{30} These observations were an important breakthrough in the understanding of the origin of GIST, indicating that they originate from the ICC or from a progenitor cell that differentiates toward a gastrointestinal pacemaker cell phenotype. The term gastrointestinal pacemaker cell tumor (GIPACT) was proposed, since it more accurately reflects current knowledge regarding differentiation than the non-committal and purely descriptive term GIST.\textsuperscript{30} The simultaneous discovery of gain-of-function mutations in the KIT gene in 5 of 6 GIST by Hirota and co-workers provided fundamental insights into the pathogenesis of GIST. Hirota et al. also showed that the mutant but not the wild type form of KIT induced malignant transformation of lymphoid cells.\textsuperscript{32}

**ICC**

The interstitial cells of Cajal (ICC), so named after the neuroanatomist Santiago Ramon y Cajal from Valencia, form a complex network of cells within the gastrointestinal tract wall. They appear to have two major functions: (1) initiating spontaneous smooth muscle contraction, i.e., pacemaker function by generating slow waves, and (2) acting as intermediaries in the control of gastrointestinal motility by the enteric nervous system as shown by their heavy innervation and junction formation with smooth muscle cells.\textsuperscript{33} Expression of the KIT proto-oncogene is essential for the development of the ICC. Disruption of KIT during development results in the absence of a functional ICC network, manifested by lack of peristalsis of the gut.\textsuperscript{34,35}

**KIT and GIST**

The KIT proto-oncogene, located on chromosome 4q11-21, is the cellular homologue of the transforming element of the Hardy-Zuckerman IV feline sarcoma virus.\textsuperscript{36-39} KIT (CD117) is a type III receptor tyrosine kinase, which belongs to the immunoglobulin supergene family.\textsuperscript{40} The molecule consists of 976 amino acids (135kDa) and is divided into an extracellular domain and an intracellular domain. The extracellular part comprises five immunoglobulin-like domains, with the second and third loops being involved in ligand binding. The intracellular tyrosine kinase domain contains a consensus ATP-binding site and a split tyrosine kinase domain (Fig. 2).\textsuperscript{40,41}
Figure 2. Schematic illustration of the structure of type III receptor tyrosine kinases. Arrows indicate the location of the gain-of-function mutations frequently found in the *KIT* or, more rarely, *PDGFRα* genes in GIST.

The ligand for KIT is stem cell factor (SCF), also known as KIT ligand (KL), Steel Factor (SF), or mast cell growth factor (MGF). Ligand binding initiates dimerization and autophosphorylation that activates downstream signaling pathways. These include the phosphatidylinositol 3-kinase (PI3K) pathway, the Ras-Raf-MAPK pathway*, and the STAT pathway, leading to proliferation, differentiation, and/or apoptosis (Fig. 3a).

A functional KIT is essential for normal development of hematopoietic cells, germ cells, melanocytes, and mast cells, as well as for the development of the interstitial cells of Cajal (ICC). Mutations in the *KIT* gene can result in a variety of diseases related to these cell types.

Gain-of-function mutations result in ligand-independent dimerization, autophosphorylation and activation of downstream signaling pathways (Fig. 3b). As previously mentioned, Hirota and co-workers reported gain-of-function mutations in exon 11 of the *KIT* gene in GIST, a finding that led to a number of studies investigating the correlation between such mutations and biological behavior. The results were, however, conflicting. While some investigators reported a strong correlation between the presence of mutations and malignant behavior, others were not able to confirm such a correlation. Most of these early studies were limited to the juxtamembrane domain, encoded by exon 11 in the *KIT* gene. However, in a few studies, the entire coding region was analyzed, leading to the detection of mutations in exon 9 and rarely exon 13 of the *KIT* gene in a small subset of GIST lacking exon 11 mutations. Rare mutations were also subsequently reported to occur in exon 17 of the *KIT* gene.

*: An interactive animation of the MAP Kinase signal transduction pathway is shown at:
http://www.bio.davidson.edu/courses/Immunology/Flash/MAPK.html
Figure 3. Activation of type III receptor tyrosine kinases. 

a: Normally, binding of the ligand induces dimerization and autophosphorylation, activating downstream signaling pathways. 
b: In GIST, gain-of-function mutations in the KIT or PDGFRα genes result in ligand-independent dimerization, autophosphorylation and activation.

Platelet derived growth factor alpha (PDGFRα) and GIST

The PDGFRα gene is located next to the KIT gene on chromosome 4q11-21. PDGFRα and KIT belong to the same family of receptor tyrosine kinases and consequently show extensive structural homology. The ligands for PDGFRα are the PDGF dimers AA, AB, BB, CC, and DD. Ligand binding initiates receptor dimerization that autophosphorylates cytoplasmic tyrosine residues (Fig. 3a) and triggers the signal transduction pathway through phosphatidylinositol 3-kinase, phospholipase Cγ, and GTPase activating proteins (reviewed in Heldin, 1992).

PDGFRα is believed to play a key role in the development of glia. Expression of PDGFRα lacking a part of the fourth and fifth Ig-like extracellular domains have been reported in primary brain tumors of glial origin.

Recently, gain-of-function mutations have been reported to occur in exons 12 and 18 of the PDGFRα gene in a subset of GIST that lack KIT mutations. KIT and PDGFRα mutations are mutually exclusive events in GIST. Tumors with PDGFRα mutations often lack immunoreactivity for KIT, have a predominantly gastric location and reportedly have a relatively benign clinical course.
**Treatment of malignant GIST**

Malignant GIST are virtually resistant to conventional chemotherapy and radiation therapy. Until recently, surgical resection has been the only available treatment. For advanced GIST, surgery alone has been inadequate and the outcome for most patients with malignant GIST has been poor.\(^7\)

A major breakthrough in the treatment of malignant GIST came in 2001 when imatinib mesylate (Glivec®, STI-571, Novartis Pharma, Basel Switzerland) was introduced as a treatment for patients with unresectable and/or metastatic GIST. Imatinib was originally designed as a treatment for chronic myelogenous leukemia (CML). In CML, imatinib blocks the binding of adenosine triphosphate to the ABL kinase in the BCR-ABL fusion gene that drives tumor proliferation. Subsequently, imatinib was found to not only inhibit ABL but also the closely related type III receptor tyrosine kinases, KIT and PDGFRα.\(^71\)

The first successful treatment of a patient with metastatic GIST with imatinib, a model of the new, rapidly growing group of molecularly targeted cancer therapies, was reported by Joensuu and co-workers in 2001.\(^72\) Imatinib thus represents the first safe and effective treatment for patients with malignant GIST, as proven by several clinical trials and ongoing clinical studies.\(^73-76\)

**Cytogenetic alterations in GIST**

Both losses and gains of chromosomes have been reported in GIST. Monosomy of chromosome 14 or partial loss of 14q are seen in up to two-thirds of tumors.\(^52,66,77-81\) At least two regions of this chromosome, 14q11.1-q12 and 14q22-24, seem to be hot spots for deletions and most likely represent sites for putative tumor suppressor genes that may play an important role in GIST development.\(^80,82\) Loss of the long arm of chromosome 22 is the second most common chromosomal aberration in GIST, detected in approximately 50% of the tumors.\(^77,78,80,83\) Losses on chromosomes 1p and 9p are less frequent, but may be more clearly associated with malignancy.\(^66,77,83-85\)

Gene amplifications have also been reported in GIST and gains on chromosome 8q and 17q have been associated with metastatic behavior.\(^80,82,84\)

**NF1 and GIST**

Neurofibromatosis type 1 (NF1), also known as von Recklinghausen’s disease, is the most common autosomal dominant inherited disorder in humans, occurring in approximately 1 in 3,500 individuals.\(^86\) It is caused by mutations in NF1, a tumor suppressor gene which has been mapped to the long arm of chromosome 17 (17q11.2).\(^87,89\) The NF1 gene is one of the largest genes in the human genome. It spans 290 kb of genomic DNA and contains 60 exons, encoding a 12-kb mRNA transcript.

Neurofibromin, the NF1 gene product, displays partial homology to GTPase activating protein (GAP), a family of RAS regulatory proteins.\(^90\) The GAP-related domain, encoded by exons 20-27a, is the only region of neurofibromin to which a biologic function has been ascribed. Neurofibromin stimulates GTPase activity and negatively regulates RAS by catalyzing the conversion of the active GTP-bound RAS to its inactive GDP-bound form. NF1 mutations disrupt the normal function of neurofibromin and result in constitutive
RAS activation. This activation increases downstream signaling through the MAP kinase pathway.

The clinical spectrum of NF1 is extremely wide and includes a plethora of organic and mental manifestations of varying degrees of severity. Most affected individuals exhibit café-au-lait spots, neurofibromas, Lisch nodules, and axillary freckling. A wide variety of neuroectodermal, neuroendocrine and mesenchymal tumors have also been associated with NF1 as well as an increased risk of developing malignancies in general.

A number of case reports and a few small series have indicated an association between NF1 and GIST. In an autopsy series of NF1 patients from Göteborg, Sweden, GIST were incidentally detected in up to one-third of the patients.
OBJECTIVES

The main objectives of this thesis were to further elucidate the phenotypic characteristics and pathogenetic mechanisms involved in sporadic as well as NF1-associated gastrointestinal stromal tumors.

The specific aims of the different studies in this thesis were:

I. to investigate the spectrum of KIT gene mutations as well as KIT isoform expression and chromosomal rearrangements in GIST in relationship to morphologic features and clinical behavior. (Paper I)

II. to study the effects of imatinib mesylate treatment in neoadjuvant, adjuvant and palliative clinical settings and to evaluate treatment response with respect to KIT mutational status. (Paper II)

III. to detect any possible correlation between different types of receptor tyrosine kinase mutations and phenotypic characteristics, risk group assessment and clinical course in a large population-based series of patients with GIST. (Paper III)

IV. to investigate the clinical and phenotypic characteristics of NF1-associated GIST and to determine mutational patterns with respect to KIT and PDGFRα in an effort to elucidate on their pathogenesis. (Paper IV)
MATERIALS AND METHODS

Tumor material
In this thesis, we have examined 259 GIST from 246 patients and 50 NF1-associated GIST from 15 NF1 patients.

Paper I: cDNA was prepared from fresh frozen tissue from 14 tumors (12 patients). Chromosome preparations were made from exponentially growing primary cultures from 10 tumors (8 patients).

Paper II: cDNA was prepared from fresh frozen tissue from 9 tumors (9 patients). Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue from 8 tumors (8 patients).

Paper III: Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue from 245 tumors (233 patients).

Paper IV: Molecular analyses were performed on genomic DNA extracted from paraffin-embedded tissue of 24 tumors (12 patients). In three patients, no material was available for molecular analyses. Immunohistochemical analyses were performed on 50 tumors (15 patients).

Methods

Denaturating high performance liquid chromatography (dHPLC)
Denaturating high performance liquid chromatography (dHPLC) is based on ion paired reverse phase chromatography. A stationary phase contained in the column is used to retain samples and a mobile phase is used to release the sample from the column. In dHPLC, the stationary phase usually consists of divinyl benzene (DVB). The mobile phase is an aqueous buffer with a mixture of acetonitrile and triethylammonium amine (TEAA). By increasing the concentration of acetonitrile, the DNA is released from the column. In contrast to regular HPLC, dHPLC is carried out at temperatures where the DNA is partially denatured.

In wild type DNA, the alleles form two homoduplexes. In samples with mutations, on the other hand, there is a mismatch and both homoduplexes and heteroduplexes are formed. Heteroduplexes have a weaker affinity for the stationary phase than homoduplexes and are therefore eluted from the column at lower concentrations of the mobile phase. Figure 4 shows a dHPLC chromatogram illustrating heteroduplexes giving rise to two extra peaks.
The major advantages of using dHPLC to screen for mutations are that: (1) it is the most sensitive method for detecting mutations, since samples with <10% mutant DNA can be detected; (2) it is a fast, cost effective, technique; and (3) it predicts the type of mutation. The major disadvantage is that it is a qualitative technique and sequencing must be performed in order to identify the mutated sequence.

**Immunohistochemical, molecular genetic, cytogenetic, and statistical methods**

The other immunohistochemical, molecular genetic and cytogenetic techniques, as well as statistical methods used in this thesis, including immunohistochemistry, PCR, cloning, nucleotide sequencing, G-banding and spectral karyotyping, are described in more detail in papers I-IV or in references therein and will not be commented on further here.
RESULTS AND DISCUSSION

The complexity of KIT gene mutations and chromosomal rearrangements and their clinical correlation in gastrointestinal stromal tumors (paper I)

KIT exon 11 mutations and biologic behavior

There have been conflicting reports regarding the effect of gain-of-function mutations in KIT on the biologic behavior of GIST. While some investigators have reported a strong correlation between mutations and a more malignant phenotype,57,59 others have not been able to confirm such a correlation.51,52

In paper I, we analyzed the mutational status of the KIT gene in 14 GIST from 12 patients. Typical gain-of-function mutations were detected in one benign tumor, in one borderline tumor, and in eight malignant tumors. The reported S715del is now known to be an alternative splice form of KIT, not a true mutation. In the four malignant GIST that lacked exon 11 mutations, no further mutations were found after analysis of the entire coding sequence of KIT. Our results indicate that the correlation between KIT mutations and biological behavior is more complex than previously reported and that alternative mechanisms driving tumor development exist.

Subsequent studies have shown that mutations also occur in exons 9, 13, and 17 of the KIT gene as well as in exons 12 and 18 of the closely related PDGFRα gene in GIST lacking KIT exon 11 mutations.66,67 The exon 9 mutations have been reported to occur particularly in small intestinal GIST and to have an aggressive behavior.103,104 Mutations in PDGFRα seem to be associated with gastric GIST of low malignant potential that frequently lack KIT immunoreactivity.66-69 These findings led us to further investigate the correlation between specific mutations types and clinical behaviors (paper III).

Cytogenetic and SKY analyses

A limited number of cytogenetic and molecular cytogenetic studies have been reported describing chromosomal rearrangements in GIST. Complete or partial loss of chromosomes 14, 22 and 1p seems to be the most common abnormalities. Using traditional G-bandning as well as spectral karyotyping (SKY analysis) for the first time in selected cases, we could confirm complete or partial losses of chromosomes 1, 14 and 22 as being the most common rearrangements in GIST. Loss of chromosome 1p was detected in 2 tumors, loss of chromosome 14 in 2 tumors, and loss of chromosome 22 in 5 tumors. We were not able to detect any correlation between chromosomal aberrations and KIT mutational status.

Later studies have indicated that loss of chromosomes 14 and 22 are very common in both benign and malignant GIST.52,66,77,81,83 Deletions of 1p and 9p are rarer and associated with malignant behavior,66,77,83,85 indicating that putative tumor suppressor genes are encoded in these regions.
GIST preferentially express the shorter, tumorigenic, isoform of exon 9 of the KIT transcript

Alternative splicing in the 3'-end of exon 9 of the KIT transcript gives rise to two isoforms of the KIT protein, GNNK+ and GNNK-. The shorter isoform, GNNK-, has been shown to induce malignant transformation when transfected into NIH3T3 cells. Preferential expression of the shorter isoform has been observed in a number of neoplasms including acute myelogenous leukemia and human germ cell tumors.

In paper I, cDNA was extracted from fresh frozen tumor tissue, enabling us to analyze the expression profile of the two isoforms in GIST. All 13 analyzed tumors showed a preferential expression of the shorter isoform. The expression pattern of normal ICC has not been analyzed; hence the biologic significance of the overexpression is unknown. Similar overexpression in other malignancies suggests, however, that it may play a role in the tumorigenesis of GIST, particularly in those tumors that lack activating KIT mutations; overexpression of the shorter isoform could serve as an alternative mechanism for increased KIT signaling. Further studies of larger series will be needed to confirm possible clinical correlations.

Neoadjuvant, adjuvant and palliative treatment of gastrointestinal stromal tumors (GIST) with imatinib: a centre-based study of 17 patients (paper II)

Gastrointestinal stromal tumors (GIST) are virtually resistant to conventional chemotherapy and radiation. Surgery has been the mainstay of treatment for malignant GIST, but surgery alone is often inadequate. Imatinib mesylate, an inhibitor of type III receptor tyrosine kinases, was originally developed to inhibit the tyrosine kinase ABL in the BCR-ABL fusion protein, driving tumorigenesis in chronic myelogenous leukemia. Lately, imatinib has been shown to have a dramatic antitumor effect in GIST, since it inhibits not only ABL, but also the closely related KIT and PDGFRα tyrosine kinase receptors. Earlier reports have indicated that specific mutation sites influence response to imatinib treatment. Preliminary studies had indicated that GIST patients with exon 11 mutations may respond better to imatinib treatment than patients whose tumors have other types of mutations or wild type KIT.

In a center-based study of 17 consecutive patients with high risk or overtly malignant GIST, we analyzed the effectiveness of imatinib in correlation to KIT exon 11 mutations. Imatinib was used in three different clinical settings - neoadjuvantly, adjuvantly, and palliatively. Clinical response to imatinib treatment correlated morphologically with tumor necrosis, fibrosis and reduced proliferative activity within the GIST. Typical gain-of-function mutations in exon 11 of the KIT gene were found in 12 of 17 patients. Missense mutations alone were found in 2 patients and deletions in 10 patients (in three patients in combination with missense mutations). Eight of 9 patients that had a partial response to imatinib had mutations. In contrast, partial response was only seen in one of the patients whose GIST lacked mutations. A KIT exon 9 mutation was later detected in this patient, a finding that may explain the partial treatment response since GIST with this mutation have been shown to respond to imatinib mesylate. None of the patients with stable or progressive disease had GIST carrying KIT exon 11 mutations.
Subsequently, treatment response has been shown to clearly correlate with the location of the mutation in *KIT* or *PDGFRα*. GIST with *KIT* exon 11 mutations have the best treatment response, while certain tyrosine kinase domain mutations (*KIT* exon 17 or *PDGFRα* exon 18) render the tumors resistant to imatinib. Thus, mutation screening of GIST has become an essential part of the clinical work up of patients.

**Gastrointestinal stromal tumors with *KIT* exon 11 deletions are associated with poor prognosis (paper III)**

Gain-of-function mutations in the *KIT* and more rarely the *PDGFRα* genes have been shown to play a critical oncogenic role in the majority of GIST. Mutations are most common in the juxtamembrane domain of *KIT*, encoded by exon 11. Clinical studies regarding the correlation of exon 11 mutations with disease outcome have been contradictory. While some investigators have indicated that GIST with exon 11 *KIT* mutations have a more aggressive course than those with wild type *KIT*, others have not. Most previous studies have lumped together the different mutation types and compared them with tumors having wild type *KIT*. Some earlier studies have, however, indicated that different types of mutations may be associated with distinctive phenotypic features and possibly also clinical behavior.

In a large population-based series of 246 GIST (from 233 patients) with long term follow-up, we determined if the mutation type among GIST with *KIT* and *PDGFRα* mutations correlated with tumor phenotype, risk group assessment and clinical course. Wild type (WT) *KIT* and *PDGFRα* were detected in 102/233 (44%) patients. *KIT* exon 11 mutations were detected in 117/233 (50%) patients; deletions were detected in 69 (30%) patients (4 of these also had a missense mutation); missense mutations were detected in 27 (12%), and duplications in 21 (9%). *KIT* exon 9 mutations were detected in 7 (7/233, 3%) patients, *PDGFRα* exons 12 and 18 mutations in 3 patients each (6/233, 2.6%), and *KIT* exon 17 mutation in one patient (1/233, 0.4%). No mutations were detected in *KIT* exon 13.

GIST with *KIT* exon 11 deletions were significantly larger in size and showed significantly more prominent histological atypia than WT tumors. Those GIST with exon 11 deletions also had a significantly higher proportion of tumors belonging to the high risk or overtly malignant risk groups and, correspondingly, a significantly greater proportion of tumors with a risk score >7 (tumor size (cm) + Ki67 max (%)) compared to the WT tumors. The deletion subgroup also had a significantly decreased disease-free survival (Fig. 5).
Using a hazard function analysis, the independent influence of KIT exon 11 deletions on disease-free survival was determined. The simultaneous influence of tumor size and proliferative index were corrected for by setting identical sizes and proliferative index values. Disease-free survival was significantly worse in GIST patients with exon 11 deletions compared to GIST patients with WT and all other types of mutations combined (p=0.0358). Disease-free survival in GIST patients with exon 11 deletions compared to those with WT tumors alone was nearly significant (p=0.0788).

Internal tandem duplications in exon 11 of the KIT gene have previously been observed in a small subgroup of GIST. These mutations cluster in the 3'-end of the exon and have been reported to be associated with gastric location, a female predominance, and usually follow a benign clinical course. In this series, we found a higher proportion of tumors with duplications (21/233, 9%) than previous reports. We did not observe any gender preference. In contrast to other reports, almost one-third of the tumors belonged to the high risk or overtly malignant risk groups and 19% of the patients died of GIST. In conclusion, duplications may be more frequent and the clinical outcome poorer than previously recognized.

Similar to previous reports, all 7 GIST in our series with KIT exon 9 mutations occurred in the small intestine. In contrast, however, we did not find the clinical course in this small group of patients to be clearly worse than GIST with WT KIT and PDGFRα. Five of the 6 patients with GIST having PDGFRα mutations belonged to the very low, low or intermediate risk groups and had benign clinical courses. Four of these were gastric and 3 were entirely or partially of epithelioid cell type. These findings correlate well with previous observations of this subset of GIST. GIST with PDGFRα mutations frequently lack immunoreactivity for KIT and the relatively low number of mutations detected in this series, particularly with regard to exon 18, may be due to the fact that immunoreactivity for KIT was used as a diagnostic criteria in the selection of cases for this population-based study.

Figure 5. Kaplan-Meier estimates of disease-free survival among 226 GIST patients with different types of KIT exon 11 mutations, KIT exon 9 duplications and WT KIT and PDGFRα. ** p=0.005 between patients with exon 11 deletions and patients with WT tumors. P values determined by log-rank test.
In conclusion, this study demonstrates that in addition to phenotypic and prognostic differences between GIST involving different receptor tyrosine kinase genes and different exon mutations, there are different subgroups of mutations within the same exon. The underlying reasons for the biological differences between GIST with different types of KIT exon 11 mutations remains unclear. The juxtamembrane domain in KIT (exon 11) normally functions to inhibit receptor dimerization in the absence of ligand. Thus, a possible explanation could be that deletions, which are clustered in the 5′-end of the exon, particularly disrupt this function.

**NF1 associated gastrointestinal stromal tumors have unique clinical, phenotypic, and genotypic characteristics (paper IV)**

There are several reports in the literature indicating that the association between NF1 and GIST is more than mere chance. A high incidence of GIST among NF1 patients has been shown in a prospective 12-year follow-up study of 70 NF1 patients; 12 of these patients were autopsied and incidental GIST were detected in 4 (33%). We studied the association between GIST and NF1 from another perspective by reviewing a consecutive series of 100 GIST patients (scrutinized for NF1) and found 5 NF1 patients. This suggests that patients with GIST carry a 150-fold increased risk of having NF1 (normal incidence approximately 1:3000). The frequent occurrence of GIST among NF1 patients suggests that NF1 patients, particularly those presenting with gastrointestinal bleeding, anemia, abdominal pain or a palpable abdominal mass, should be clinically investigated for GIST.

A total of 50 macroscopically detected NF1-associated GIST from 15 NF1 patients were histologically reviewed and immunohistochemically analyzed. We found that NF1-associated GIST have a unique phenotype. In addition to a propensity for multicentricity within the gastrointestinal tract, detected in 9/15 patients (3 to >100 tumors), their distribution deviates from that of sporadic GIST. The 15 NF1 patients in this series had GIST involving the jejunum and/or ileum and 4 had duodenal tumors; only 4 had gastric tumors and all of these were also associated with small intestinal tumors. The NF1-associated GIST had a predominantly spindle cell morphology. Only 3 of 50 histologically examined tumors in this series were partially of the epithelioid cell type; the remainders were entirely spindled. This is in contrast to sporadic GIST in which one-third of tumors are partly or entirely of the epithelioid type. Also striking is the almost uniformly histological low-grade appearance of the NF-associated GIST. A unique finding in this series was the occurrence of focal or diffuse ICC hyperplasia in the vicinity of the primary tumor as well as within distant separate foci of the gastrointestinal wall. The striking ICC hyperplasia seen in our cases of NF1-associated GIST is also highly suggestive of a precursor lesion for GIST.

Activating, oncogenic KIT mutations have been reported in 50-80% of sporadic GIST in most series. Recently, one-third of GIST with wild type KIT have been found to have activating PDGFRA mutations. KIT and PDGFRA mutations seem to be mutually exclusive. In contrast, no such mutations were detected in KIT or PDGFRA in our series of 24 NF1-associated GIST. Thus, this study shows that NF1-associated GIST are genotypically distinct from sporadic GIST. This is the first large series unequivocally demonstrating the absence of PDGFRA mutations in NF1-associated GIST. Two
recently reported NF1-associated GIST with wild type \textit{KIT} were also found to lack \textit{PDGFRA} mutations.\cite{98} Our findings further support the contention that the underlying pathogenesis of NF1-associated GIST differs from most sporadic GIST.

In summary, the incidence of GIST is markedly increased in NF1 patients. NF1-associated GIST are unique with regard to multicentricity, predominant location in the small bowel, spindle cell morphology and lack of \textit{KIT} and \textit{PDGFRA} mutations. In contrast to sporadic GIST, the vast majority of NF1-associated GIST have a favorable clinical course.
CONCLUSIONS

The main observations and conclusions of this thesis can be summarized as follows:

I. The role of KIT mutations in the biological behavior of GIST is more complex than previously recognized. A malignant phenotype can occur without activating mutations and mutations may occur in clinically benign tumors.

Losses of whole or parts of chromosomes are the most common cytogenetic abnormalities in GIST, suggesting that loss of multiple chromosomal regions, presumably containing putative tumor suppressor genes, is an important genetic event in GIST.

GIST preferentially express the shorter, tumorigenic isoform (GNNK-) of KIT exon 9. Preferential overexpression of this isoform could be significant in tumors lacking KIT and PDGFR-A mutations and serve as an alternative mechanism for increased KIT signaling.

II. Imatinib mesylate is an effective treatment of malignant GIST, particularly for patients whose tumors harbor KIT exon 11 mutations.

III. The type of KIT exon 11 mutation influences phenotype, biological behavior and clinical outcome in GIST.

Patients with tumors having KIT exon 11 deletions have a significantly poorer prognosis than patients with tumors having other mutation types or wild type KIT and PDGFR-A.

In addition to tumor size and proliferative index, KIT exon 11 deletion is an independent adverse prognostic factor in GIST.

IV. There is a strong association between GIST and NF1.

NF1 associated GIST represent a unique subset of GIST that typically are multicentric, of low malignant potential and appears to originate from hyperplastic foci of interstitial cells of Cajal (ICC). The lack of activating mutations in the KIT and PDGFR-A genes in NF1 associated GIST indicates that different pathogenetic mechanisms are involved.
FUTURE PERSPECTIVES

Gene expression analyses

The completion of the human genome (HUGO) project and recent important technical breakthroughs have made large scale gene expression profiling feasible. Recently, distinct gene expression profiles have been detected in GIST with KIT and PDGFRα mutations. These findings may be of clinical importance and may lead to the development of highly selective therapeutic targets. Similarly, studies of gene expression profiles in GIST with different subtypes of KIT exon 11 mutations could potentially help to explain the prognostic differences among GIST subgroups and be of future therapeutic importance.

Using oligo microarray, we will analyze the expression profiles of different subgroups of GIST. These subgroups include tumors of different risk groups; tumors with or without mutations in the KIT and PDGFRα genes; tumors with mutations in different exons of KIT or PDGFRα; and tumors with different types of mutations in exon 11 of the KIT gene. Our aim is to gain further understanding of the pathogenetic mechanisms involved in the development and progression of GIST.

NF1 gene alterations

NF1 associated GIST are genotypically and phenotypically distinct from sporadic GIST, suggesting that the pathogenetic mechanisms underlying tumor development differ. There is a connection between KIT/PDGFRα and NF1. Neurofibromin, encoded by the NF1 gene, is a negative regulator of RAS. RAS is involved in the MAPK signaling pathway that can be triggered by activation of KIT or PDGFRα. High levels of KIT expression have also been detected in neurofibromin-deficient Schwann cells derived from a neurofibroma of an NF1-patient. This is in contrast to non-NF1 associated Schwann cells, which do not express KIT.

In paper IV, we examined the mutation status of the KIT and PDGFRα genes in a rather large series of NF1 associated GIST. Using the same NF1-associated as well as sporadic GIST, we will expand the study and investigate the NF1 gene for mutations or other aberrations such as loss of heterozygosity (LOH) with the aim to identify pathogenetic mechanisms involved in tumor development and progression in this subset of GIST. The possibility of somatic NF1 mutations in sporadic GIST will also be explored.

Splice isoform expression

The shorter isoform of KIT exon 9 has been shown to be tumorigenic. Preferential overexpression of this isoform is seen in human malignancies such as myelogenous leukemia and human germ cell tumors. In paper I, we reported the same expression profile in GIST. The biological significance is unknown, but it may serve as an alternative mechanism for KIT activation in GIST that lack activating mutations. We would like to extend the analysis of the expression pattern of the two isoforms using a large series of GIST, including tumors of all risk groups and tumors with different mutational status, in
order to further elucidate the biological and clinical relevance of the preferential isoform expression.

Chromosomal rearrangements

Losses of parts of or whole chromosomes frequently occur in GIST. Loss of 14 and 22 are the most common and are believed to be early events in the tumor development. Loss of 1p and 9, in contrast, occur later and have been associated with malignant behavior, indicating that the deleted regions may contain putative tumor suppressor genes.

The INK4a locus, encoding the $p16^{INK4a}$ and the $p14^{ARF}$ genes on chromosome 9p21, is frequently inactivated in malignant tumors. Using our unique population-based material, we will study losses of chromosomes or parts of chromosomes in GIST. In particular, the study will focus on loss of chromosome 9p, encoding the INK4a locus, since deletions of this region have been reported to be associated with malignant behavior. Our aim is to further elucidate receptor tyrosine kinase mutations and pathogenetic mechanisms involved in the progression of GIST.

Mutation type specific treatment response to imatinib mesylate in GIST

Imatinib mesylate has been shown to be an effective drug treatment for patients with malignant GIST. The response differs, however, with regard to the mutation status of the $KIT$ or $PDGFR\alpha$ genes. Tumors with mutations in $KIT$ exon 11 are more responsive to imatinib mesylate than tumors with other mutations or with WT $KIT$ and $PDGFR\alpha$. Some mutations (in $KIT$ exon 17 and $PDGFR\alpha$ exon 18) render GIST resistant to drug treatment with imatinib mesylate.

In paper III, we showed that not only the location of the mutation, but also the type of mutation within the exon influence the biological behaviour of GIST. Tumors with $KIT$ exon 11 deletions are associated with a decreased disease-free survival. Whether the effectiveness of imatinib mesylate treatment varies within subgroups of GIST with $KIT$ exon 11 mutations – deletions, missense mutations and duplications - is unclear. Information regarding the possible correlation between mutation type status and treatment response could be of value in identifying which patients will benefit most from treatment with imatinib mesylate.
POPULÄRVETENSKAPLIG SAMMANFATTNING

Under de senaste decennierna har det blivit klarlagt att cancer är en genetisk sjukdom. Ca 90 % av alla tumörer orsakas av att fel uppstått i en eller, i de flesta fall, flera gener. Människans arvsmassa (DNA) består av ca 25000 gener. Generna kodar för proteiner som bygger upp och utför cellens processer. Cellers tillväxt (proliferation) är normalt strikt kontrollerad, men ibland brister denna kontroll och en tumör (cancer) kan börja utvecklas. En cancercell är en från början normal cell, som genom en ansamling av fel i arvsmassan fått förmågan att växa okontrollerat och invadera omgivande vävnad (metastasera). Dessa fel, s.k. mutationer, drabbar ofta gener som har som funktion att kontrollera proliferation, apoptos (celldöd) eller att reparera skador i arvsmassan. Det finns tre huvudtyper av mutationer; deletioner - som innebär att en del av genen försvunnit, substitutioner - som innebär att en byggsten i DNA bytts ut mot en annan och duplikationer - som innebär att en del av genen dubblerats.

Tumörer som uppstår i ben och mjukdelar kallas sarkom. Till gruppen sarkom räknas gastrointestinala stromacellstumörer (GIST), som denna avhandling fokuserar på. GIST är den vanligaste formen av gastrointestinala sarkom och uppkommer ofta i magsäcken, men förekommer i hela mag-tarmkanalen. Ungefär 15 individer per miljon invånare och år drabbas av GIST. Den är en relativt heterogen tumörtyp och det kliniska förloppet varierar kraftigt mellan olika patienter. Ungefär en tredjedel av tumörerna är små och långsamtväxande, de ger sällan symptom utan upptäcks ofta av en lump i samband med andra undersökningar. Prognosen för patienter med denna typ av GIST är relativt god. Resterande två tredjedelar är stora och snabbväxande tumörer som oftast har en dålig eller mycket dålig prognos. Vad som orsakar de nåra variation är inte helt klarlagt.

Det huvudsakliga syftet med denna avhandling har varit att studera dels hur vanliga mutationer är i två olika gener i GIST, dels hur dessa mutationer påverkar det biologiska beteendet hos tumörerna. Generna, som heter KIT och PDGFRA (platelet derived growth factor receptor alpha), fungerar som en slags gaspedal för cellen, som när de aktiveras får signaler att börja dela sig. Gener av denna typ kallas proto-onkogener. I många olika typer av tumörer är dessa gener muterade, vilket leder till att de blir överaktiv och övergår till att bli onkogener.


I det tredje arbetet fortsatte vi studera betydelsen av mutationer i KIT och PDGFRA i GIST. Materialet bestod av 233 GIST patienter och det är en av de största serier som undersöks samt den första populations-baserade studien av GIST. Vi fann att drygt hälften av patienterna hade mutationer; vanligast var deletioner i en specifik del av KIT genen (exon 11). För första gången kunde vi visa att tumörer med deletioner har en signifikant sämre prognos än tumörer med andra mutationstyper eller tumörer som saknar
mutationer. Tumörer med deletioner hade högre tillväxt och var signifikant större. Tumörstorlek och proliferationshastighet har tidigare visats vara oberoende prognostiska faktorer för GIST. I denna studie kunde vi visa att även deletioner i KIT exon 11 är en oberoende faktor för dålig prognos, oavsett tumörstorlek och proliferationshastighet.

GIST är i stort sett helt resistenta mot strålning och kemoterapi (cellgifter) och den enda behandlingsformen har länge varit kirurgi. Tyvärr kan man inte operera alla patienter, beroende på tumörens utbredning, och prognosen för dessa var tidigare mycket dålig. De senaste åren har detta kommit att ändras sedan ett nytt läkemedel, imatinib mesylat (Glivec), introducerats. Imatinib mesylat inaktiverar onkogenerna KIT och PDGFR_ och resultatet blir att tumörens tillväxt avstannar. I vårt andra arbete studerade vi om det fanns ett samband mellan behandlingsvar och förekomst av mutationer i KIT i en serie av 17 GIST patienter. Vi kunde visa att så är fallet; det är de patienter som har mutationer i exon 11 som svarar bäst på behandling. Åtta av de 9 patienter som svarade bra hade mutationer, men det hade ingen av de patienter som inte svarade på behandlingen.

Neurofibromatos typ 1 (NF1) är en av de vanligaste ärfliga sjukdomarna. Patienter med NF1 drabbas oftare än normalbefolkningen av olika tumörer, bl.a. GIST. I det sista arbetet studerade vi GIST som uppstår hos NF1 patienter och jämförde dem med sporadiska GIST, d.v.s. från patienter som inte har NF1. Materialbestod av 50 GIST tumörer från 15 NF1 patienter, och är en av de största serier som publicerats. Våra resultat visar att NF1 associerade GIST är en unik undergrupp av GIST. Patienterna har ofta många tumörer som framför allt uppkommer i tunntarm och det kliniska förloppet är godartat. Även genetiskt skiljer sig NF1 associerade GIST från sporadiska GIST i det att de saknar mutationer i KIT och PDGFR. Detta tyder på att de mekanismer som driver tumörutvecklingen är annorlunda.

Vi har visat att den biologiska betydelsen av KIT mutationer i GIST är mer komplex än vad man tidigare trott och att mutationstypen påverkar kliniskt beteende och behandlingssvar med imatinib mesylat. NF1 associerade GIST är en unik undergrupp av GIST som saknar KIT och PDGFR mutationer och har ett benigne kliniskt förlopp.
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Wherever you are it is your friends who make your world.
/William Jones
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